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Another new species of *Dixonius* (Squamata, Gekkonidae) from Gia Lai Province in the Central Highlands, Vietnam

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Abstract

Another new species of *Dixonius*, *D. fulbrighti* **sp. nov.**, is described from Gia Lai Province, in the Central Highlands of Vietnam, using an integrated approach based on morphological, categorical (color pattern), and mitochondrial NADH dehydrogenase subunit 2 gene (ND2) and its flanking tRNAs data. Phylogenetic analyses recovered *Dixonius fulbrighti* **sp. nov.** as closely related to *D. gialaiensis* from Gia Lai Province and part of a clade that includes *D. minhlei*, *D. siamensis*, and *D. somchanhae*. Multivariate (PCA, DAPC, and MFA) and univariate (ANOVA) analyses of 15 meristic (scale counts), six morphometric (mensural), and five categorical (color pattern and morphology) characters from 44 specimens of all eight species of *Dixonius* from Vietnam, Laos, and Cambodia clearly demonstrated that *Dixonius fulbrighti* **sp. nov.** is statistically different and diagnostically distinct from all closely related species of *Dixonius*. This new species discovery highlights the underestimated gecko diversity and the importance of continued fieldwork in the Central Highlands of Vietnam.

Key Words

Integrative taxonomy, new species, leaf-toed gecko, morphology, molecular phylogeny, Indochina, Southeast Asia

Introduction

The Central Highlands of Vietnam are composed of basaltic, quartzite, and granite substrates, and are mainly situated at elevations ranging from 800–2400 m, forming the central and southern parts of the Truong Son Mountain Range (Sterling et al. 2006). This upland mountain system is composed of "sky islands" that are isolated from each other by vast intervening lowlands, which have contributed to the evolution of unique and distinct forms (Bain and Hurley 2011). Unfortunately, these regions are undergoing habitat loss due to the conversion of natural forests into agricultural farmland (Luu et al. 2020; as observed by Vinh Quang Luu). Previous studies on gecko species have mainly been focused in protected areas (e.g., Nazarov et al. 2008; Jestrzemski et al. 2013; Do et al. 2021) but our recent research indicates that geckos recently described in the Central Highlands are also found outside the network of protected areas (Luu et al. 2017, 2023). There is still much to be learned about the mechanisms that contribute to the high levels of diversity within this morphologically and ecologically diverse gecko lineage.

In Vietnam, *Dixonius* species are distributed in the southern regions from Gia Lai to Dong Nai provinces, with seven known species, including *D. aaronbaueri* Ngo & Ziegler, 2009 from Ninh Thuan and Binh Thuan provinces; *D. gialaiensis* Luu, Nguyen, Le, Grismer, Ha, Sitthivong, Hoang & Grismer, 2023; *D. melanostictus* (Taylor, 1962) from Dong Nai Prvince (Nguyen et al. 2009); *D. minhlei* Ziegler, Botov, Nguyen, Bauer, Brennan, Ngo & Nguyen, 2016 from Dong Nai Province; *D. siamensis* (Boulenger, 1898) from Ninh Thuan, Gia Lai, Lam Dong,

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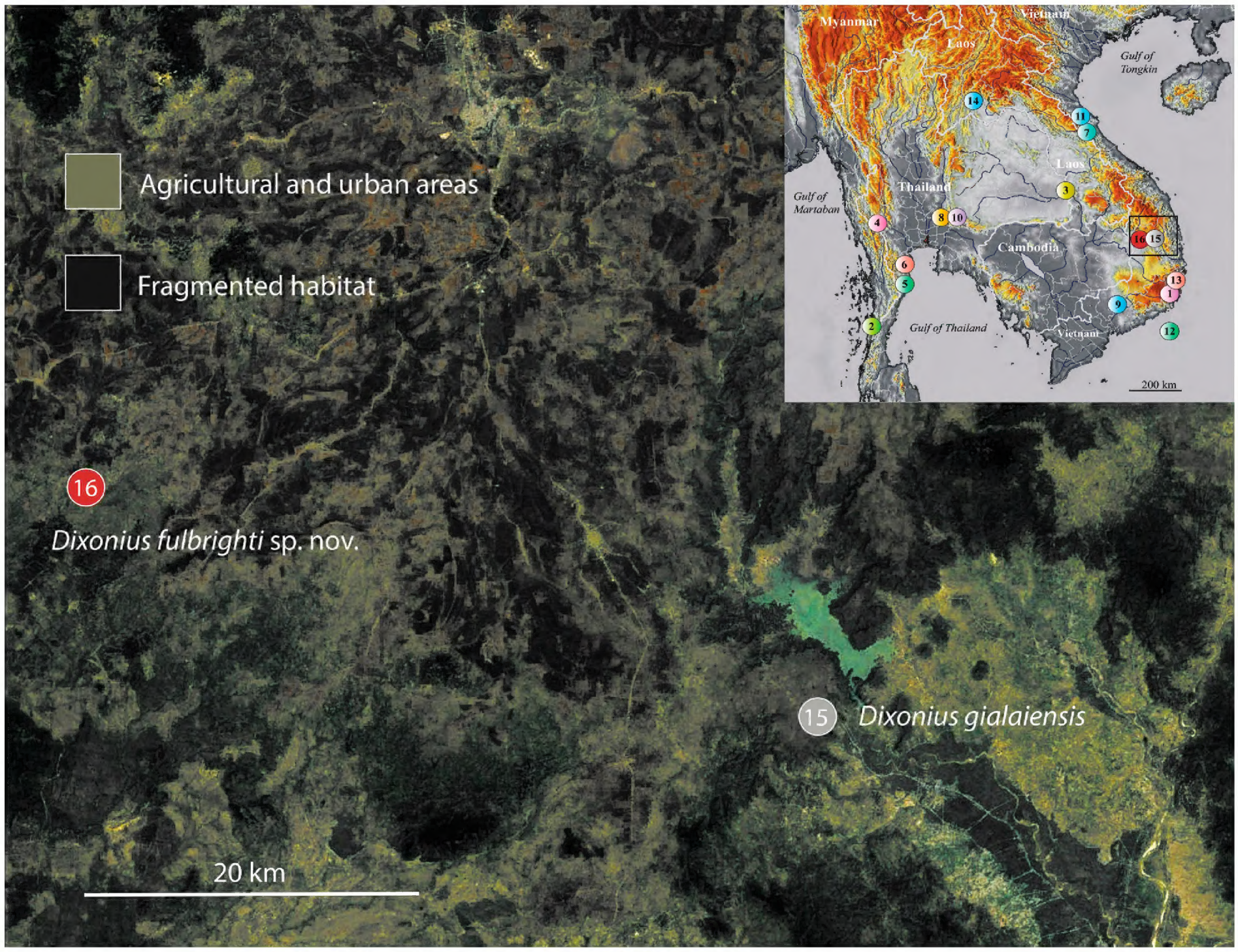

Figure 1. Location of the type localities of all known species of *Dixonius* (**1** *Dixonius aaronbaueri* from Ninh Thuan Province, Vietnam; **2** *D. dulayaphitakorum* from Ranong Province, Thailand; **3** *D. mekongensis* from Ubon Ratchathani Province, Thailand; **4** *D. hangseesom* from Kanchanaburi Province, Thailand; **5** *D. kaweesaki* from Prachuap Khiri Khan Province, Thailand; **6** *D. pawangkhananti* from Phetchaburi Province, Thailand; **7** *D. lao* from Khammouane Province, Laos; **8** *D. melanostictus* from Nakhon Ratchasima Province, Thailand; **9** *D. minhlei* from Dong Nai Province, Vietnam; **10** *D. siamensis* from SaraBuri and Nakhon Ratchasima provinces; **11** *D. somchanhae* from Vientiane Capital, Laos; **12** *D. taoi* from Binh Thuan Province, Vietnam; **13** *D. vietnamensis* from Khanh Hoa Province, Vietnam; **14** *D. muangfuangensis* from Vientiane Province, Laos; **15** *D. gialaiensis* from Gia Lai Province, Vietnam; **16** *D. fulbrighti* sp. nov. from Gia Lai Province, Vietnam. The inset delimits the study area.

Binh Phuoc provinces (Nguyen et al. 2009); *D. taoi* Botov, Phung, Nguyen, Bauer, Brennan & Ziegler, 2015 from Binh Thuan Province; and *D. vietnamensis* Das, 2004 from Khanh Hoa and Binh Thuan provinces.

While exploring geckonid diversity in the Central Highlands of Vietnam, a recent survey recovered four specimens of *Dixonius* from the Ia Grieng Commune, Duc Co District, Gia Lai Province (Fig.1). Based on phylogenetic evidence from the mitochondrial NADH dehydrogenase subunit 2 (ND2) gene and flanking tRNAs, morphometric, meristic, and color pattern data, they could not be assigned to any known species and are therefore described below as new species.

Materials and methods

A total of four *Dixonius* specimens were caught by hand from Duc Co town, Duc Co District, Gia Lai Province, Vietnam. The specimens were fixed in approximately 80% ethanol and then transferred to 70% ethanol for permanent storage. Liver tissue samples taken before the specimens were preserved were stored separately in 95 % ethanol. The specimens were deposited in the collection of the Vietnam National University of Forestry (VNUF), Hanoi, Vietnam.

Species delimitation

The general lineage concept (GLC: de Queiroz 2007) adopted herein proposes that a species constitutes a population of organisms evolving independently from other such populations owing to a lack of, or limited gene flow. By "independently," it is meant that new mutations arising in one species cannot spread readily into another species (Barraclough et al. 2003; de Queiroz 2007). Molecular phylogenies recovered multiple monophyletic mitochondrial lineages of individuals (populations) that were used

to develop initial species-level hypotheses – the grouping stage of Hillis (2019). Discrete color pattern data and univariate and multivariate analyses of morphological data were then used to search for characters and morphospatial patterns consistent with the tree-designated species-level hypotheses – the construction of boundaries representing the hypothesis-testing step of Hillis (2019) – thus providing independent diagnoses to complement the molecular analyses. In this way, delimiting (phylogeny) and diagnosing (taxonomy) species are not conflated (Frost and Hillis 1990; Frost and Kluge 1994; Hillis 2019).

Molecular data and phylogenetic analyses

Three samples (VNUF R.2022.81 (field number GL22.01), VNUF R.2022.82 (field number GL22.02), and VNUF R.2022.84 (field number GL22.04)) of the newly collected specimens were analyzed. We used the protocols of Nguyen et al. (2021) for DNA extraction, amplification, and sequencing. The complete NADH dehydrogenase subunit 2 (ND2) gene with six partial or complete adjacent tRNAs, approximately 1200 bp long, respectively, were amplified and sequenced using the primer pair, MetF6(5'-AAGCAGTTGGGCCCATACC-3') and COIR1(5'-AGRGTGCCAATGTCTTTGTGRTT-3') (Macey et al. 1997).

Genomic DNA was extracted from all liver tissues stored in ethanol following the standard protocols of DNeasy blood and tissue kit, Qiagen (California, USA). The PCR volume consisted of 20µl (1µl each primer, 7µl water, 10µl of Taq mastermix and 1 µl DNA template). PCR conditions were: 95 °C for 5min, followed by 42 cycles: 95 °C for 30s, 50 °C for 45s and 72 °C for 60s with a final elongation step for 6 min at 72 °C. PCR products were visualized using electrophoresis through a 1.2% agarose gel, marker 100 bp, 1X TAE and stained with RedSafe Nucleic Acid Staining Solution and photographed under UV light of Geldoc system (Quantum CX5, Villber, France). Successful amplifications were purified using innuPREP Gel Extraction Kit (Analytik Jena, Germany). Cleaned PCR products were sent to Genewiz from Azenta Life Sciences for sequencing in both directions.

We obtained 1,431 base pairs from the NADH dehydrogenase subunit 2 gene (ND2) and the flanking tRNAs from 32 ingroup samples of *Dixonius* representing 14 nominal species, including the new samples from Gia Lai Province Vietnam. *Heteronotia spelea* was used as an outgroup to root the trees following Gamble et al. (2015). Sequence data for the other species were downloaded from GenBank. Newly generated sequences were deposited in GenBank (Table 1).

Maximum Likelihood (ML) and Bayesian Inference (BI) were used to estimate phylogenetic trees. Best-fit models of evolution determined in IQ-TREE (Nguyen et al. 2015) using the Bayesian information criterion (BIC) implemented in ModelFinder (Kalyaanamoorthy et al.

Table 1. Specimens used for the phylogenetic analyses.

Species	Catalog no.	Location	GenBank no.
Dixonius aaronbaueri	ZFMK87274	Nui Chua NP, Ninh Thuan Province, southern Vietnam	HM997152
Dixonius fulbrighti sp. nov.	VNUF R.2022.81 (Field no. GL.01)	Duc Co District, Gia Lai Province, Vietnam	OR327037
Dixonius fulbrighti sp. nov.	VNUF R.2022.82 (Field no. GL.02)	Duc Co District, Gia Lai Province, Vietnam	OR327038
Dixonius fulbrighti sp. nov.	VNUF R.2022.84 (Field no. GL.04)	Duc Co District, Gia Lai Province, Vietnam	OR327039
Dixonius gialaiensis	VNUF R.2020.22	Chu Se District, Gia Lai Province, Vietnam	OQ819041
Dixonius gialaiensis	VNUF R.2020.33	Chu Se District, Gia Lai Province, Vietnam	OQ819042
Dixonius lao	VNUF R.2016.2	Khammouane Province, Laos	MT024681
Dixonius lao	IEBR A.2019.5	Khammouane Province, Laos	MT024683
Dixonius lao	IEBR A.2019.6	Khammouane Province, Laos	MT024682
Dixonius melanostictus	VU 022	Captive, Thailand	HM997153
Dixonius minhlei	ZFMK 97745	Vinh Cuu, Dong Nai Province, Vietnam	KX379194
Dixonius muangfuangensis	VNUF R.2020.42 (Field no. MF02)	Muangfuang District, Vientiane Province, Central Laos	OQ818586
Dixonius muangfuangensis	VNUF R.2020.52 (Field no. MF03)	Muangfuang District, Vientiane Province, Central Laos	OQ818587
Dixonius cf. *siamensis*	VU 023	Captive, Thailand	KX379195
Dixonius siamensis	LSUHC 7328	Phnom Aural, Purset Province, Cambodia	EU054299
Dixonius siamensis	FMNH 263003	Keo Seima District, Mondolkiri Province, Cambodia	EU054298
Dixonius siamensis	LSUHC 7378	Phnom Aural, Purset Province, Cambodia	KP979732
Dixonius somchanhae	VNUF R.2020.2	Nasaithong District, Vientiane Capital, Laos	MW605166
Dixonius somchanhae	VNUF R.2020.1	Nasaithong District, Vientiane Capital, Laos	MW605165
Dixonius somchanhae	VNUF R.2020.3	Nasaithong District, Vientiane Capital, Laos	MW605167
Dixonius somchanhae	VNUF R.2020.55 (Field no. VT05)	Vientiane Capital, Laos	OQ818589
Dixonius somchanhae	VNUF R.2020.54 (Field no. VT04)	Vientiane Capital, Laos	OQ818588
Dixonius somchanhae	VNUF R.2020.59 (Field no.VT09)	Vientiane Capital, Laos	OQ818591
Dixonius somchanhae	VNUF R.2020.56 (Field no. VT0T06)	Vientiane Capital, Laos	OQ818590
Dixonius sp.	LSUHC 9466	Sai Yok, Kanchanaburi Province, Thailand	KX379196
Dixonius taoi	ZFMK 96680	Phu Quy Island, Binh Thuan Province, Vietnam	KP979733
Dixonius taoi	CAS 257300	Phu Quy Island, Binh Thuan Province, Vietnam	KP979734
Dixonius taoi	IEBR A 2014-26	Phu Quy Island, Binh Thuan Province, Vietnam	KP979735
Dixonius taoi	IEBR A 2014-27	Phu Quy Island, Binh Thuan Province, Vietnam	KP979736
Dixonius cf. *vietnamensis*	ZFMK 87273	Nui Chua, Ninh Thuan Province, Vietnam	KX379201
Dixonius vietnamensis	IEBR R.20163	Nha Trang, Khánh Hòa Province, Vietnam	KX379198

2017) indicated that F81+F was the best-fit model of evolution for the tRNAMET and K2P+I, and HKY+F+G4 were the best models of evolution for tRNAs2 and ND2, respectively. The ML analysis was performed using the IQ-TREE webserver (Trifinopoulos et al. 2016) with 1000 bootstrap pseudoreplicates using the ultrafast bootstrap (UFB) analysis (Minh et al. 2013; Hoang et al. 2018). The BI analysis was performed on CIPRES Science Gateway (Miller et al. 2010) using MrBayes v3.2.4 (Ronquist et al. 2012) using default models of evolution. Two independent runs were performed using Metropolis-coupled Markov Chain Monte Carlo (MCMCMC), each with four chains: three hot and one cold. The MCMCMC chains were run for 80,000,000 generations with the cold chain sampled every 8000 generations and the first 10% of each run being discarded as burn-in. The posterior distribution of the trees from each run were summarized using the sumt function in MrBayes v3.2.4 (Ronquist et al. 2012). Stationarity was checked in Tracer v1.6 (Rambaut et al. 2014) to ensure effective sample sizes (ESS) for all parameters were well above 200. We considered Bayesian posterior probabilities (BPP) of 0.95 and above and ultrafast bootstrap support values (UFB) of 95 and above as an indication of strong nodal support (Huelsenbeck et al. 2001; Minh et al. 2013). Uncorrected pairwise sequence divergences (p-distance) were calculated in MEGA 11 (Kumar et al. 2016) using the complete deletion option to remove gaps and missing data from the alignment.

A time-calibrated Bayesian phylogenetic tree was estimated using BEAST 2 (Bayesian Evolutionary Analysis by Sampling Trees) version 2.7.3 (Drummond et al. 2012) implemented in CIPRES (Cyberinfrastructure for Phylogenetic Research; Miller et al. 2010) where the ingroup node subtending the split between *Dixonius aaronbaueri* and the remaining species was given a 24.04 mya prior with an offset range of 20.23–27.68 mya following Gamble et al. (2015). The split between *Heteronotia* and *Dixonius* was dataed at 45.0 mya with an offset range of 33.3–56.8 mya (Gamble et al. 2015). An input file was constructed in BEAUti (Bayesian Evolutionary Analysis Utility) version 2.7.3. An optimised relaxed clock with unlinked site models, linked clock and tree models, using a Calibrated Yule prior were employed for the species level analysis. BEAST Model Test (Bouckaert and Drummond 2017), implemented in BEAST, was used to numerically integrate over the uncertainty of substitution models while simultaneously estimating phylogeny using Markov chain Monte Carlo (MCMC). MCMC chains were run for 60 million generations and logged every 6,000 generations. The BEAST log file was visualized in Tracer v. 1.7.2 (Rambaut et al. 2014) to ensure effective sample sizes (ESS) were above 200 for all parameters. A maximum clade credibility tree using mean heights at the nodes was generated using TreeAnnotator v.2.7.3 (Rambaut and Drummond 2013) with a burnin of the first 10% of each run. Nodes with Bayesian posterior probabilities (BPP) of 0.95 and above were considered strongly supported (Hulsenbeck et al. 2001; Wilcox et al. 2002).

Morphological data and analysis

The morphological data set comprised four individuals of the new *Dixonius* population from Duc Co District, Gia Lai Provine, Vietnam (VNUF R.2022.81-84) and seven closely related species based on the phylogeny (see below), including three type specimens of *D. gialaiensis* from Gia Lai Province, Vietnam (VNUF R.2020.22, 2020.33, 2020.44), six type specimens of *D. minhlei* from Dong Nai Province, Vietnam (IEBR A.0801-02, VNMN R.2016.1-2, ZFMK 97745-46), three type specimens of *D. muangfuangensis* from Vientiane Province, Laos (VNUF R.2020.42, NUOL R.2022.01, VNUF R.2020.52), three type specimens of *D. lao* from Khammouane Province, Laos (VNUF R.2016.2, IEBR A.2016.5-6), eight specimens of *D. siamensis* from Pursat Province, Cambodia (LSUHC 07328, 07378, 08487, 08491, 08522, 09284, 09289), five type specimens of *D. somchanhae* from Vientiane Capital, Laos (VNUF R.2020.1-5), and 12 specimens of *D. vietnamensis* from Nha Trang Province, Vietnam (ZRC 2.6024-27, IEBR R.2016.1, 2016.3, 2016.4, VNMN R.2016.3-4, ZFMK 97747-49).

Morphometric and meristic data were taken from the 44 specimens following Bauer et al. (2004) and Ngo and Ziegler (2009). Morphometric characters were measured after preservation with a digital caliper to the nearest 0.1mm and a zoom stereomicro scope on the right/left of the body and included SVL: snout-vent length (taken from the tip of the snout to the vent), TaL: tail length (taken from the vent to the tip of the tail, original or partially regenerated), TW: tail width (taken at the base of the tail immediately posterior to the postcloacal swelling), BW: body width (greatest width of torso, taken at the level of midbody), HL: head length (the distance from the posterior margin of the retroarticular process of the lower jaw to the tip of the snout), HW: head width (measured at the angle of the jaws), HD: head depth (the maximum height of head measured from the occiput to base of the lower jaw), EL: ear length (greatest oblique length across the auditory meatus), TBL: Tibia length (taken on the ventral surface from the posterior surface of the knee while flexed 90° to the base of the heel), AG: axilla to groin length (taken from the posterior margin of the forelimb at its insertion point on the body to the anterior margin of the hind limb at its insertion point on the body), FA: forearm length (taken on the ventral surface from the posterior margin of the elbow while flexed 90° to the inflection of the flexed wrist), ED: eye diameter (the greatest horizontal diameter of the eye-ball), EN: eye nostril distance (measured from the anterior margin of the bony orbit to the posterior margin of the external nares), ES: eye snout distance (measured from anteriormost margin of the bony orbit to the tip of snout), EE: eye ear distance (measured from the anterior edge of the ear opening to the posterior margin of the eye-ball), IN: internarial distance (measured between the external nares across the rostrum), IO: interorbital distance (measured between the dorsal-most edges of the bony orbits).

Meristic characters included V: ventral scales (counted transversely across the abdomen midway between limb insertions from one ventrolateral fold to the other), DTR: longitudinal rows of dorsal tubercles (counted transversely

across the body midway between the limb insertions from one ventrolateral body fold to the other), PV: paravertebral scales (counted in a paravertebral row from first scale posterior to parietal scale to last scale at the level of vent opening), PV': paravertebral scales (counted in a row between limb insertions), T4: lamellae under fourth toe (from the distal scale containing claw to basal scale that broadly contacts adjacent fragmented scales), IOS: Interorbital scales (counted at narrowest point between orbits across the frontal bone), ICS: interciliary scales (counted between supraciliaries at midpoint of orbit), SPL: supralabials (counted from the largest scale at the corner of the mouth to the rostral scale), IFL: infralabials (counted from termination of enlarged scales at the corner of the mouth to the mental scale), MO: number of supralabial at midorbital position, PP: number of precloacal pores in males.

Color pattern characters on dorsum included the presence or absence of canthal stripes (CanthStrp), presence or absence of strong darkly barred lips (LipBar), presence or absence of dark-colored round blotches on the top of the head (RdHdBlch) and dorsum (RdBodBlch), and presence or absence of two regularly arranged rows of whitish tubercles on flanks (Tub). The raw morphological data for all characters and specimens are presented in Tables 2, 3.

Table 2. Sex and raw meristic and categorical color pattern data used in the analyses from specimens of *Dixonius* from Vietnam and Laos. m = male; f = female; j = juvenile; r/l = right/left.

Species	Museum no.	Sex	Meristic data						Categorical data				
			SPLr/l	IFLr/l	MO	IOS	V	T4r/l	Canthal stripe	Lips strongly barred	Blotches on the head round	Blotches on dorsum round	Two rows of regularly disposed whitish tubercles on each side
D. fulbrighti sp. nov.	VNUF R.2022.81	m	8	6.5	6	9	24	14	present	no	no	yes	present
D. fulbrighti sp. nov.	VNUF R.2022.82	f	7.5	5.5	6	8	23	14.5	present	no	no	yes	present
D. fulbrighti sp. nov.	VNUF R.2022.83	fj	8	7	6	8	23	14	present	no	no	yes	present
D. fulbrighti sp. nov.	VNUF R.2022.84	f j	8.5	6	6	8	22	13.5	present	no	no	yes	present
D. minhlei	IEBR A.0802	m	8	6	6	10	22	14	present	no	yes	yes	absent
D. minhlei	ZFMK 97746	m	8	6.5	6	10	23	14.5	present	no	yes	yes	absent
D. minhlei	IEBR A.0801	f	8.5	7	6	10	22	12	present	no	yes	yes	absent
D. minhlei	ZFMK 97745	f	7.5	6	5.5	10	23	13	present	no	yes	yes	absent
D. minhlei	VNMN R.2016.1	f	8	6	5.5	8	23	15	present	no	yes	yes	absent
D. minhlei	VNMN R.2016.2	f	8	6.5	6	7	20	13	present	no	yes	yes	absent
D. gialaiensis	VNUF R.2020.22	m	7.5	6	6	7	21	14	present	yes	yes	yes	present
D. gialaiensis	VNUF R.2020.33	f	7	6	6	7	19	14	present	yes	yes	yes	present
D. gialaiensis	VNUF R.2020.44	mj	8	7	6	7	21	14.5	present	yes	yes	yes	present
D. vietnamensis	ZRC 2.6024	m	5	6	5	10	20	13	present	no	no	no	present
D. vietnamensis	ZRC 2.6025	m	5	6	5	9	20	13	present	no	no	no	present
D. vietnamensis	ZRC 2.6026	j	5	6	6	8	20	13	present	no	no	no	present
D. vietnamensis	ZRC 2.6027	j	6	7	6	8	20	13	present	no	no	no	present
D. vietnamensis	IEBR R.2016.3	m	8	6	5.5	10	19	13.5	present	no	no	no	present
D. vietnamensis	VNMN R.2016.3	m	7.5	6	5.5	9	19	13.5	present	no	no	no	present
D. vietnamensis	IEBR R.2016.1	f	7	6	5.5	8	18	13.5	present	no	no	no	present
D. vietnamensis	VNMN R.2016.4	f	7.5	7	6	9	20	13	present	no	no	no	present
D. vietnamensis	ZFMK 97748	f	7.5	6	6	8	20	14	present	no	no	no	present
D. vietnamensis	ZFMK 97747	mj	7.5	6	5.5	10	15	13.5	present	no	no	no	present
D. vietnamensis	IEBR R.2016.4	f j	8	7	6	7	21	12.5	present	no	no	no	present
D. vietnamensis	ZFMK 97749	fj	7	6.5	5.5	8	19	13.5	present	no	no	no	present
D. somchanhae	VNUF R.2020.3	m	7	5	6	8	24	14	present	yes	no	no	present
D. somchanhae	VNUF R.2020.2	m	8	6	6	8	23	15	present	yes	no	no	present
D. somchanhae	VNUF R.2020.1	m	8	5.5	6	8	23	15	present	yes	no	no	present
D. somchanhae	VNUF R.2020.4	f	8	5.5	6	8	23	15	present	yes	no	no	present
D. somchanhae	VNUF R.2020.5	f	8	6	6	7	26	13	present	yes	no	no	present
D. siamensis	LSUHC09284	f	8	7	6	9	19	14	absent	yes	no	yes	present
D. siamensis	LSUHC08522	f	8	6.5	6	10	22	14.5	absent	yes	no	yes	present
D. siamensis	LSUHC08487	f	8	7	6	10	20	14.5	absent	yes	no	yes	present
D. siamensis	LSUHC08420	m	8.5	7	6	10	21	13	absent	yes	no	yes	present
D. siamensis	LSUHC08491	f	8	7	6	9	20	14.5	absent	yes	no	yes	present
D. siamensis	LSUHC07328	j	7.5	6	5.5	9	22	14	absent	yes	no	yes	present
D. siamensis	LSUHC07378	m	8	6	6	10	20	14.5	absent	yes	no	yes	present
D. siamensis	LSUHC09289	m	7.5	6	6	10	21	16	absent	yes	no	yes	present
D. muangfuangensis	NUOL R.2022.01	m	7	6.5	6	7	21	15	absent	yes	no	no	present
D. muangfuangensis	VNUF R.2020.42	m	8	7	6	7	20	15	absent	yes	no	no	present
D. muangfuangensis	VNUF R.2020.52	f	8	6.5	6	7	21	15	absent	yes	no	no	present
D. lao	VNUF R.2016.2	m	9.5	8	7.5	9	23	15	absent	yes	no	no	absent
D. lao	IEBR A.2019.5	f	8.5	8	7	8	23	15	absent	yes	no	no	absent
D. lao	IEBR A.2019.6	f	9	7.5	8	8	24	15	absent	yes	no	no	absent

Table 3. Sex and raw morphometric data that were normalized and used in the analyses from specimens of *Dixonius* from Vietnam and Laos. m = male; f = female; j = juvenile; r/l = right/left.

Species	Museum no.	Sex	SVL	BW	HL	HW	HD	EL	ED	EN	ES	EE	IN	IO	FAr	TBLr	AGr
D. fulbrighti sp. nov.	VNUF R.2022.81	m	46	10.7	13.7	7.8	5.7	1.4	2.2	3.4	4.8	3.7	1.7	1.9	5.5	6.8	20.2
D. fulbrighti sp. nov.	VNUF R.2022.82	f	35.2	7.5	10.9	6.9	3.3	1.1	2.1	2.9	4.1	3.2	1.3	1.6	4.7	6.2	14.2
D. fulbrighti sp. nov.	VNUF R.2022.83	fj	31.1	5.1	9.2	5.1	2.4	1.2	2.2	2.3	2.3	2.5	1.1	1.4	4.5	5.1	12.5
D. fulbrighti sp. nov.	VNUF R.2022.84	f j	30.3	5.6	9.3	5.8	3	0.8	2	2.7	3.4	2.9	1.1	1.5	3.7	4.6	13.3
D. minhlei	IEBR A.0802	m	43.9	9.4	7.3	7.7	4.7	1.5	2.7	3.7	5	3.5	1.6	4	6.2	7.7	18.7
D. minhlei	ZFMK 97746	m	40.6	8.5	6.7	6	4.3	1.3	2.2	3.2	4.4	3.6	1.3	3.5	6.7	7	18.2
D. minhlei	IEBR A.0801	f	45.9	9.7	7.2	6.6	5.2	1.2	3.3	3.4	4.4	3.4	1.5	3.7	5.9	7.2	21.2
D. minhlei	ZFMK 97745	f	47.5	9.6	7.6	6.8	4.7	1.5	3.1	3.5	4.9	3.9	1.5	3.7	6	7.3	21.5
D. minhlei	VNMN R.2016.1	f	43.3	9.3	7.1	6.5	4.4	1.3	2.5	3.5	4.6	3.8	1.5	3.8	6.1	7.5	20.6
D. minhlei	VNMN R.2016.2	f	46.7	9.2	7.7	6.2	4.6	1.2	3.1	3.8	5.2	3.6	1.5	3.4	6.6	7	30.3
D. gialaiensis	VNUF R.2020.22	m	41.2	8.6	11.7	7.7	5.2	1.1	2.9	3.1	4.3	3.3	1.3	1.2	6.1	6.9	15.8
D. gialaiensis	VNUF R.2020.33	f	47.4	8.4	12.3	8.8	6.1	1.3	3.3	3.5	4.8	3.5	1.5	1.4	6.3	7.7	21.8
D. gialaiensis	VNUF R.2020.44	mj	35.9	8.3	10.9	6.8	4.7	0.9	2.6	2.9	3.8	3	1.3	1.3	4.5	5.6	14
D. vietnamensis	ZRC 2.6024	m	40.8	8	7.5	7.9	5.5	1	2.9	3.2	4.3	3.8	2.1	3.6	5.6	7.7	21
D. vietnamensis	ZRC 2.6025	m	42.4	9.1	7.5	7.6	6	1.1	2.9	3.7	4.6	4	1.6	3.6	6.2	7.2	21
D. vietnamensis	ZRC 2.6026	j	26.6	5.4	5.4	5.2	4	0.6	2.1	2.2	3	2.5	1.1	2.7	4.4	4.4	13
D. vietnamensis	ZRC 2.6027	j	25.9	4	5.2	5.1	3.3	0.6	1.8	2.3	3.5	2.2	0.9	2.1	4	4.6	11.8
D. vietnamensis	IEBR R.2016.3	m	39	6.5	6.9	6.8	4.2	1.1	2.8	2.9	3.9	3.1	1.1	1.6	4.7	6.5	14.8
D. vietnamensis	VNMN R.2016.3	m	39.9	7.8	7.2	7	4.7	0.8	2.5	3.4	4.6	3.3	1.3	1.7	5.2	6.5	16.6
D. vietnamensis	IEBR R.2016.1	f	43.5	7.6	7.6	6.9	4.7	1	2.7	3.1	4.5	2.7	1.3	1.6	5	6.2	19.2
D. vietnamensis	VNMN R.2016.4	f	43.7	8.6	7.7	7.7	4.7	1.1	2.8	3.4	4.7	3.6	1.3	1.8	5.5	6.3	18.2
D. vietnamensis	ZFMK 97748	f	45.2	10.3	8.5	8.2	5.7	1.3	2.9	3.8	5.4	4.3	1.5	2.7	5.5	6.6	19.2
D. vietnamensis	ZFMK 97747	mj	34.1	4.7	6.2	5.7	4.1	0.9	2.3	2.7	3.6	2.5	1.2	1.3	4.2	5.9	12.3
D. vietnamensis	IEBR R.2016.4	f j	31.2	5.2	5.7	5.8	3.7	0.9	2.4	2.4	3.4	2.4	1	1.2	3.1	4.8	11.9
D. vietnamensis	ZFMK 97749	fj	29.2	4.8	5.2	4.8	3.1	0.9	2.5	2.1	2.9	2.3	1	1.2	3.1	4.9	11.1
D. somchanhae	VNUF R.2020.3	m	43.8	9.4	12.2	8.5	5.6	1.6	3.4	3	5.1	3.4	1.3	1.7	6.2	7.3	20.5
D. somchanhae	VNUF R.2020.2	m	47.1	11.1	12.9	9.7	5.9	1.9	3.3	3.4	5	3.5	1.6	1.8	5.6	8	19.5
D. somchanhae	VNUF R.2020.1	m	39.8	8.9	11.6	7.9	5.2	1.2	2.9	2.9	4.2	3.1	1.7	1.3	4.8	6.6	17.4
D. somchanhae	VNUF R.2020.4	f	35.5	9.4	9.7	6.9	4.2	1.2	2.2	2.8	3.7	2.5	1.2	1.4	4.3	5.5	15.5
D. somchanhae	VNUF R.2020.5	f	39.9	8.9	11.4	7.6	4.4	1.5	3.1	2.6	4	3.1	1.5	1.3	4.9	6	19.7
D. siamensis	LSUHC09284	f	45.4	8.6	12.8	8.7	5.2	1.6	3	3.7	5.1	4	2	3.6	6.7	7.3	19
D. siamensis	LSUHC08522	f	44.1	9.4	12.5	8.1	5.7	1.4	2.4	4.4	5.2	4.5	1.8	3.7	6.7	6.9	21.2
D. siamensis	LSUHC08487	f	48.6	10.7	14.3	8.7	5.4	1.6	3.2	3.4	5.4	4.2	1.7	3.5	7.1	8	21.8
D. siamensis	LSUHC08420	m	46.9	8.8	13.1	9.1	5.3	1.3	2.7	3.7	5.3	3.9	1.5	3.7	6.7	7.3	20.7
D. siamensis	LSUHC08491	f	45.2	10.2	13	8.2	5.7	1.4	2.8	3.3	4.7	4.2	2	3.7	6.2	6.9	19
D. siamensis	LSUHC07328	j	28.6	5.8	8.4	5.5	3	0.7	2.1	2.4	3.3	2.8	1.5	2.8	3.8	5	12
D. siamensis	LSUHC07378	m	36.7	6.5	10.9	7.3	4.5	1.3	2.6	3.1	4.6	3.4	1.6	3.4	6	6.6	16.1
D. siamensis	LSUHC09289	m	45.3	9.1	12.7	8.6	5.1	1.6	2.6	3.7	5	3.6	2	3.5	7	7.3	18.9
D. muangfuangensis	NUOL R.2022.01	m	38.3	7.83	10.5	7.2	4.3	0.8	2.4	2.8	3	3.4	1.3	1.7	4.3	4.9	16.5
D. muangfuangensis	VNUF R.2020.42	m	55.6	11.93	15.2	10.8	6.9	2.3	3	3.8	5.9	5.1	1.6	2.3	6.8	7.2	23.1
D. muangfuangensis	VNUF R.2020.52	f	56.7	12.23	16.7	10.7	6.9	2.1	3.5	3.8	5.8	5.1	1.7	2.4	7.1	7.3	27.4
D. lao	VNUF R.2016.2	m	50.1	9.7	14.1	9.2	5.3	1.4	3.6	4.4	5.6	4.1	1.7	1.7	6.9	7.6	20.6
D. lao	IEBR A.2019.5	f	55.4	11.5	14.3	9.7	6.2	1.7	3.6	4	5.5	4.4	1.8	1.5	7.1	8.5	22.2
D. lao	IEBR A.2019.6	f	35.8	7.2	9.9	7	4	1.1	2.7	2.8	3.6	2.6	1.1	1.1	4.6	5.9	15.2

All morphological statistical analyses were performed using R v.4.2.1 (R Core Team, 2021). Morphometric characters used in the statistical analyses were SVL, BW, HL, HW, HD, EL, ED, EN, ES, EE, IN, IO, FAr, TBLr, and AGr. Tail metrics were not used due to the high degree incomplete sampling (i.e. regenerated, broken, or missing). To remove potential effects of allometry on morphometric traits (sec. Chan and Grismer 2022), we used the following equation: $Xadj = \log(X) - \beta[\log(SVL) - \log(SVLmean)]$, where Xadj=adjusted value; X=measured value; β=unstandardized regression coefficient for each population; and SVLmean=overall average SVL of all populations (Thorpe 1975, 1983; Turan 1999; Lleonart et al. 2000, accessible in the R package GroupStruct (available at https://github.com/chankinonn/GroupStruct). The morphometrics of each species were normalized separately and then concatenated into a single data set so as not to conflate potential intra- with interspecific variation (Reist 1986; McCoy et al. 2006). All data were scaled to their standard deviation to ensure they were analyzed on the basis of correlation and not covariance. Meristic characters (scale counts) used in the statistical analyses were SPLr/l, IFLr/l, MO, IOS, ICS, V, DTR, and T4r/l. Paried meristics were averaged if they differed. Precloacal and femoral pores were omitted from the multivariate analyses due to their absence in females. Categorical color pattern characters analyzed were CanthStrp, LipBar, RdHdBlch, RdBodBlch, and Tub.

Analyses of variance (ANOVA) were conducted on meristic and normalized morphometric characters (see below) with statistically similar variances – following a Levene's test; $p > 0.05$ – to search for the presence of statistically significant mean differences ($p < 0.05$) among species across the data set. Following the ANOVAs, each data set was subjected to a TukeyHSD test to ascertain which species pairs differed significantly from each other for which particular characters. Boxplots were generated for discrete meristic characters in order to visualize the range, mean, median, and degree of differences between pairs of species bearing statistically different mean values and violin plots were generated for continuous morphometric characters to visualize the same.

Morphospatial positions were visualized using principal component analysis (PCA) from the ADEGENET package in R (Jombart et al. 2010) on the normalized and meristic data to determine if lineage positioning was consistent with the putative species boundaries delimited by the molecular phylogenetic analyses and defined by the univariate analyses (see above). PCA, implemented using the "prcomp()" command in R, is an unsupervised analysis plotting the overall variation among individuals (i.e. data points) while treating each individual independently (i.e. not coercing data points into pre-defined groups). Subsequent to the PCA, a discriminant analysis of principle components (DAPC) was used to test for corroboration and further discrimination of morphospatial differences among the putative species. DAPC *a priori* groups the individuals of each predefined population inferred from the phylogeny into separate clusters (i.e., plots of points) bearing the smallest within-group variance that produce linear combinations of centroids having the greatest between-group variance (i.e. linear distance; Jombart et al. 2010). DAPC relies on standardized data from its own PCA as a prior step to ensure that variables analyzed are not correlated and number fewer than the sample size. Principal components with eigenvalues accounting for 90–95% of the variation in the data set were retained for the DAPC analysis according to the criterion of Jombart et al. (2010).

To test and further corroborate the PCA and DAPC analyses, we conducted a multiple factor analysis (MFA) on the above mentioned morphological characters plus the categorical color pattern characters for a near total evidence data set (see Tables 5, 6). The MFA was implemented using the *mfa*() command in the R package *FactorMineR* (Husson et al. 2017) and visualized using the *Factoextra* package (Kassambara and Mundt 2017). MFA is a global, unsupervised, multivariate analysis that incorporates qualitative and quantitative data (Pagès 2015), making it possible to analyze different data types simultaneously in a nearly total evidence environment. In a MFA, each individual is described by a different set of variables (i.e. characters) which are structured into different data groups in a global data frame – in this case, quantitative data (i.e. meristics and normalized morphometrics) and categorical data (i.e. color pattern). In the first phase of the analysis, separate multivariate analyses are carried out for each set of variables – principal component analyses (PCA) for the quantitative data sets and multiple correspondence analysis (MCA) for categorical data. The data sets are then normalized separately by dividing all their elements by the square root of their first eigenvalues. For the second phase of the analysis, the normalized data sets are concatenated into a single matrix for a global PCA of the data. Standardizing the data in this manner prevents one data type from overleveraging another. In other words, the normalization of the data in the first phase prevents data types with the most number of characters or the greatest amount of variation from outweighing other data types in the second phase. This way, the contribution of each data type to the overall variation in the data set is scaled to define the morphospatial distance between individuals as well as calculating each data type's and each character's contribution to the overall variation in the data set (Pagès 2015; Kassambara and Mundt 2017).

Results

Molecular results

The ML, BI, and BEAST analyses yielded trees with identical topologies and strong support at almost every node (see Fig. 2). The molecular analyses revealed that *Dixonius aaronbaueri* is the sister species to a clade containing all other *Dixonius* species. Additionally, all analyses supported the newly discovered population from Duc Co District, Gia Lai Province, Vietnam, as the well-supported sister species (1.00/100) of the clade containing *D. minhlei* and *D. gialaiensis* (see Figs 2, 3). The uncorrected pairwise sequence divergences among *Dixonius* species ranged from 2.57% to 18.84% (see Table 4). The new population from Duc Co District, Gia Lai Province, had a range of 3.12% to 16.43% and is most similar to *D. gialaiensis* and most distant from *D. aaronbaueri*.

The time-calibrated BEAST analysis suggests Vietnam's lineages, the population from Duc Co District, Gia Lai Province and *D. gialaiensis–D. minhlei* clade diverged from each other approximately 4.12 mya (1.84–6.79 HPD) (see Fig. 3).

Statistical analyses

The first two principal components (PC1 and PC2) of the PCA analysis recovered 56.6% of the variation in the morphometric and meristic data set (see Fig. 4A). These components loaded most heavily for body width (BW), head width (HW), eye nostril distance (EN), eye snout distance (ES), and eye ear distance (EE) along PC1, and interorbital distance (IO), supralabials (SPLr/l), number of supralabial at midorbital position (MO), and ventral scales

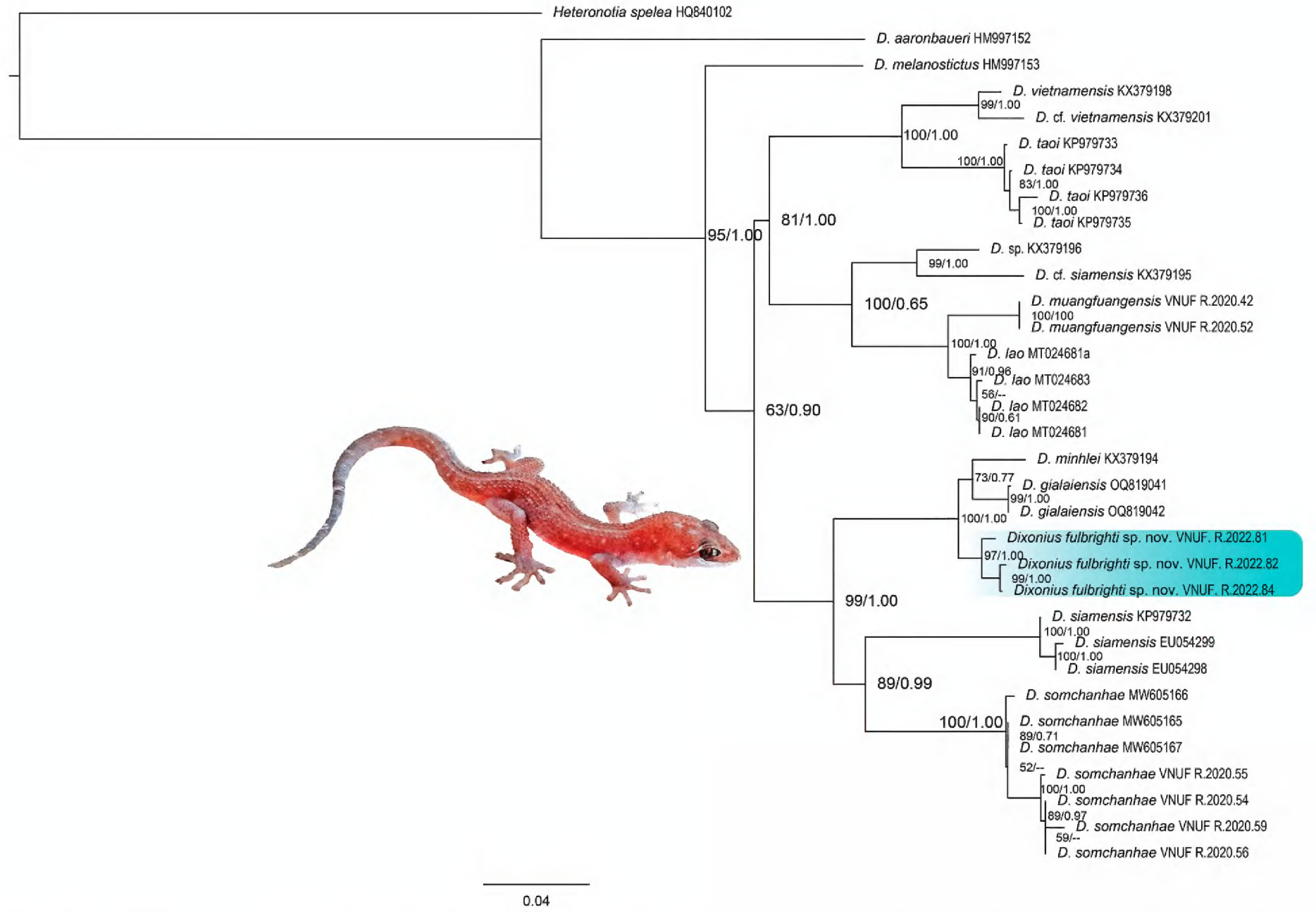

Figure 2. Maximum likelihood topology with ultrafast bootstrap values (UFB) and Bayesian posterior probabilities (BPP) at the nodes.

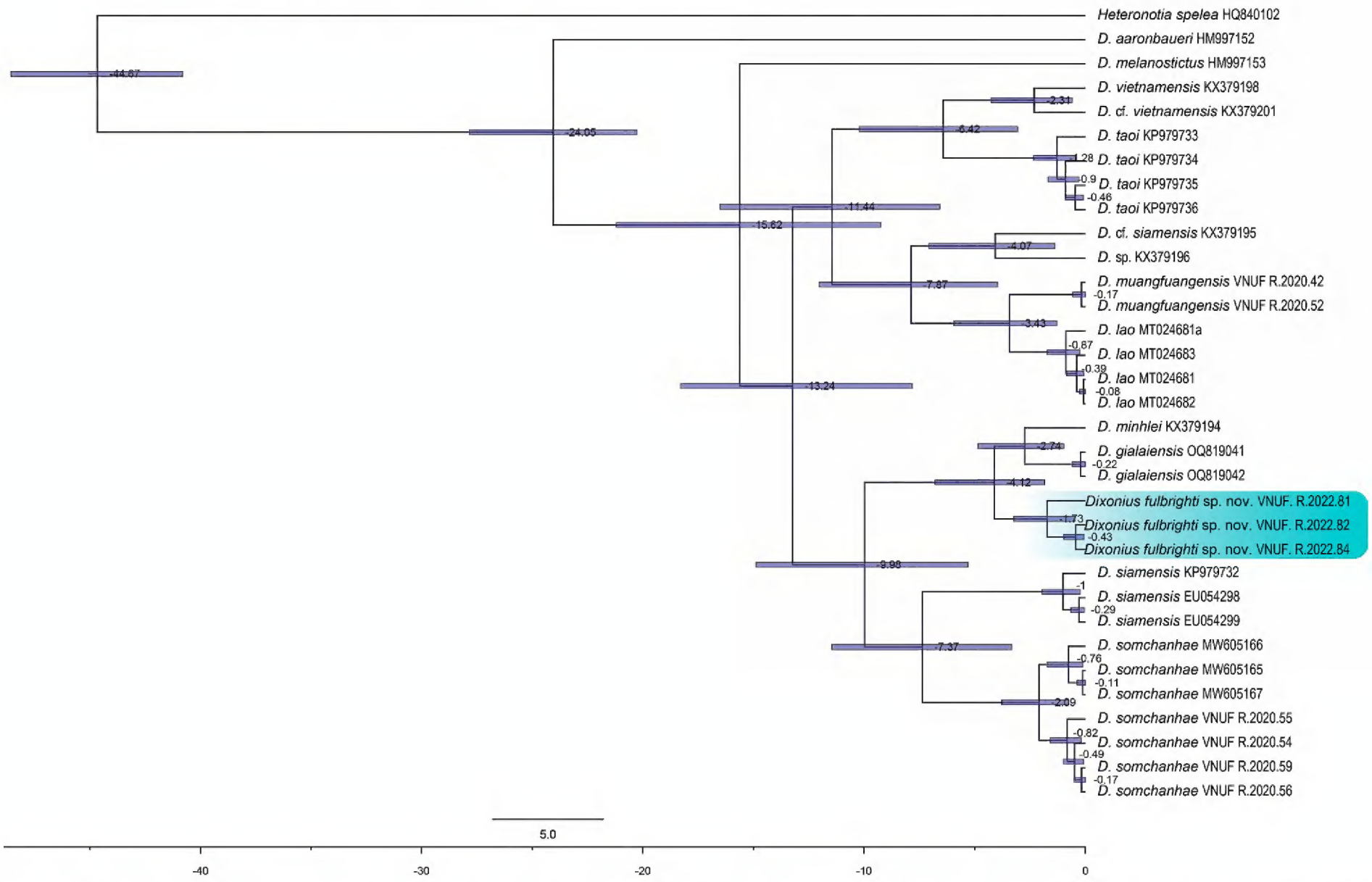

Figure 3. BEAST chronogram of the *Dixonius* species from Vietnam and Laos. Numbers at the nodes are mean ages in millions of years. Bars represent the 95% highest posterior densities.

Table 4. Mean percentages of uncorrected pairwise sequence divergence (p-distances) among the species of *Dixonius*. Intraspecific p-distance are in bold font, n/a = data not applicable.

	***D. fulbrighti* sp. nov.**	***D.* sp.**	***D.* cf. *siamensis***	***D. aaronbaueri***	***D. taoi***	***D. vietnamensis***	***D.* cf. *vietnamensis***	***D. muangfuangensis***	***D. lao***	***D. minhlei***	***D. gialaiensis***	***D. siamensis***	***D. somchanhae***	***D. melanostictus***
D. fulbrighti sp. nov.	**0.01**													
D. sp.	13.11	**n/a**												
D. cf. *siamensis*	14.15	6.33	**n/a**											
D. aaronbaueri	16.43	18.52	18.37	**n/a**										
D. taoi	13.27	11.87	13.54	16.60	**0.01**									
D. vietnamensis	13.09	12.12	13.67	18.84	6.96	**n/a**								
D. cf. *vietnamensis*	12.90	12.12	12.43	18.31	7.74	2.57	**n/a**							
D. muangfuangensis	12.85	10.78	8.17	18.17	11.74	12.79	12.50	**0.00**						
D. lao	11.54	8.63	9.43	16.76	11.66	12.07	11.56	3.27	**0.00**					
D. minhlei	3.61	13.97	15.33	17.56	13.73	14.13	13.92	13.23	13.41	**n/a**				
D. gialaiensis	3.12	13.56	14.32	15.68	12.20	13.44	13.22	13.15	11.20	3.65	**0.00**			
D. siamensis	10.29	13.80	14.92	16.56	12.68	12.42	12.32	12.80	12.36	12.64	10.61	**0.00**		
D. somchanhae	10.65	13.61	13.12	18.05	13.62	12.91	12.74	12.94	12.82	12.59	11.01	9.57	**0.00**	
D. melanostictus	11.48	13.30	13.04	15.16	11.83	13.12	13.01	11.99	10.87	14.09	11.57	12.41	11.43	**n/a**

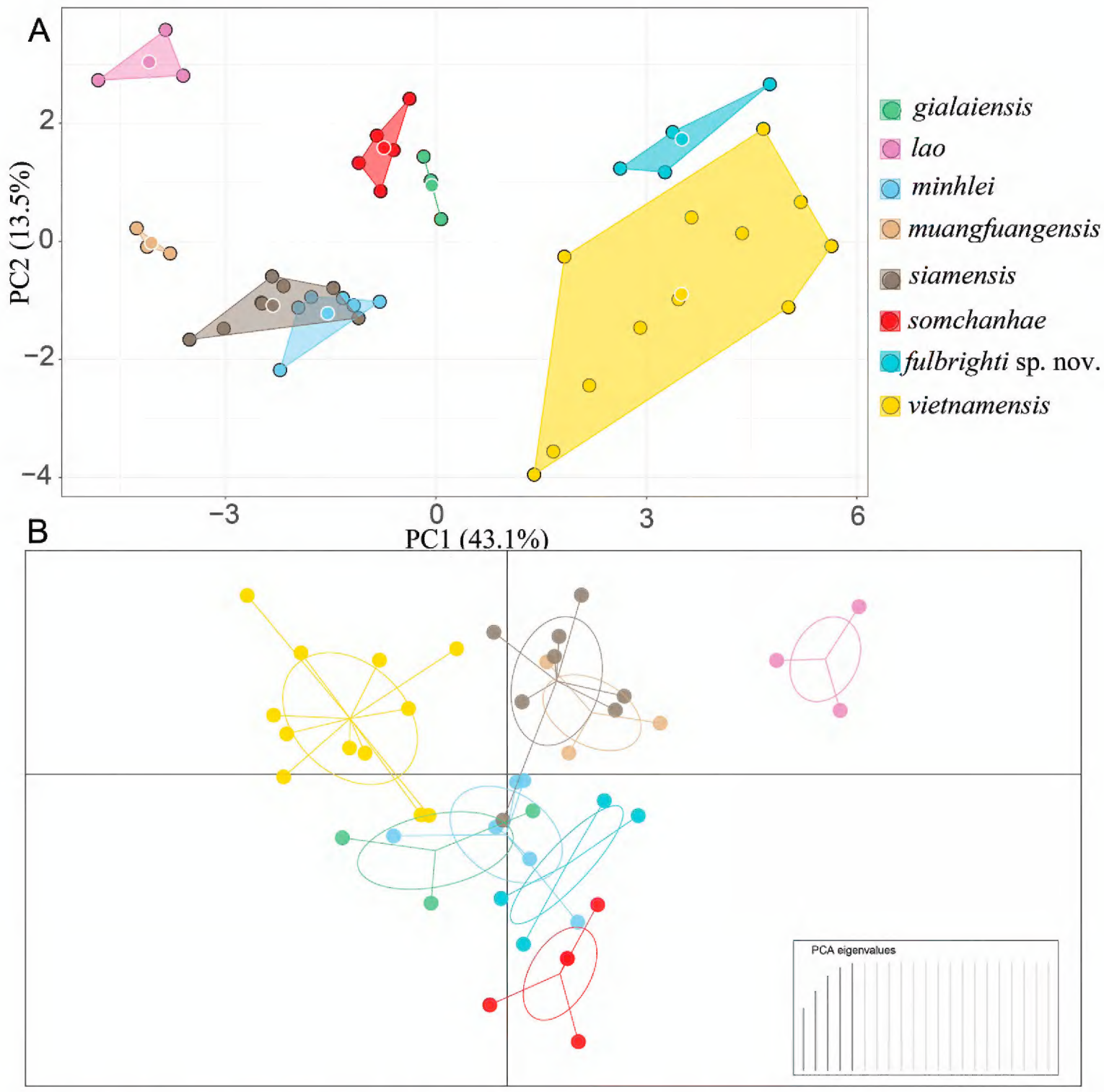

Figure 4. A Principal component analysis (PCA) of *Dixonius* species based on the morphometric and meristic data showing their morphospatial relationships along the first two components. B Discriminant analysis of principal components (DAPC) based on retention of the first five PCs with 67% inertia ellipsoids.

Table 5. Summary statistics and principal component analysis scores for *Dixonius* species. Abbreviations are listed in the Materials and methods.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Standard deviation	3.01003227	1.685877698	1.23949927	1.189032683	1.136318219	0.950656207	0.922402779
Proportion of Variance	0.43144	0.13534	0.07316	0.06732	0.06149	0.04304	0.04052
Cumulative Proportion	0.43144	0.56678	0.63994	0.70727	0.76875	0.81179	0.85231
eigen	9.060294267	2.842183612	1.53635844	1.413798721	1.291219096	0.903747223	0.850826887
SVL	-0.183137642	0.011423135	-0.069418522	0.076025214	-0.119546371	0.451176774	-0.589642945
BW	-0.287276767	0.064974951	0.187163981	-0.199453201	0.019777911	-0.133068566	-0.114357041
HL	-0.222534372	0.251387029	0.23514022	0.300194841	0.119329084	-0.056134295	0.150350725
HW	-0.264923454	0.100856053	0.274888978	0.257153297	-0.193697896	-0.025433828	0.20364848
HD	-0.239223187	-0.126312635	0.224210506	-0.024761051	-0.413575793	0.029259717	0.233903564
EL	-0.2480955	0.169750915	0.178082873	0.002208353	0.210266124	0.137233441	-0.232577889
ED	-0.202876478	0.122593123	0.079950567	-0.239727042	-0.47584928	0.235373894	-0.048044975
EN	-0.265593548	-0.130857091	-0.293077474	0.03772842	0.029146276	-0.105240363	0.142115916
ES	-0.267303156	-0.128737264	-0.102433066	-0.036514974	-0.068578553	-0.009743264	0.195931987
EE	-0.276238196	-0.150072094	-0.016576264	0.149081788	0.006956725	-0.271219422	-0.084638951
IN	-0.239210846	-0.181935095	0.070874242	0.114696597	0.170327297	0.022503069	0.138746934
IO	-0.131045671	-0.460675273	-0.164479294	-0.032535496	0.242758327	-0.169473493	-0.152061581
FAr	-0.279019143	-0.171574811	-0.122828868	-0.090763378	0.096076353	-0.023168457	0.04857391
TBLr	-0.256167278	-0.099347048	-0.096744886	-0.219547386	0.043230096	0.332696024	0.101539921
AGr	-0.262180808	-0.1304743	0.000207287	-0.261650023	-0.044763987	-0.216118201	-0.26353247
SPLr.l	-0.138456955	0.383331303	-0.225322477	0.206591458	0.113507526	0.110869199	-0.176892801
IFLr.l	-0.089464182	0.168661032	-0.585083828	0.180041929	-0.237863864	-0.199925005	-0.127174583
MO	-0.156905954	0.393579439	-0.305813247	0.010226561	-0.089299256	-0.164453012	0.254918394
IOS	-0.068091843	-0.230600144	0.078301763	0.673617186	0.047911701	0.164434462	-0.062199104
V	-0.140473075	0.310134826	0.27924011	-0.11493361	0.255279707	-0.405751544	-0.23957707
T4r.l	-0.152382721	0.157971329	-0.130436885	-0.183960387	0.490226326	0.396201891	0.316382072
	PC8	**PC9**	**PC10**	**PC11**	**PC12**	**PC13**	**PC14**
Standard deviation	0.843138943	0.710443326	0.614017867	0.525772586	0.515133085	0.463343505	0.418149629
Proportion of Variance	0.03385	0.02403	0.01795	0.01316	0.01264	0.01022	0.00833
Cumulative Proportion	0.88616	0.91019	0.92815	0.94131	0.95395	0.96417	0.97249
eigen	0.710883277	0.50472972	0.37701794	0.276436812	0.265362095	0.214687204	0.174849113
SVL	0.265288193	-0.446853384	0.196416397	-0.110376596	0.139472173	-0.13673416	0.085656549
BW	0.09057594	-0.129906618	-0.173199899	0.177854897	0.108703018	-0.085524495	-0.103253386
HL	-0.070645969	-0.213980283	-0.087846741	-0.389903504	-0.321747003	-0.17842853	-0.021701889
HW	0.039378469	-0.022086396	0.070374143	0.022285485	0.134276418	-0.209651757	0.058151643
HD	0.139854316	-0.180680891	-0.127550531	0.157737189	-0.027411952	0.137530567	-0.134451774
EL	-0.203202698	0.278604397	-0.198153958	0.402630535	-0.109054652	-0.264792874	0.164044114
ED	-0.319474696	0.251272882	-0.008164379	0.059050615	-0.034208937	0.225488518	0.158104491
EN	0.295981088	0.09576584	0.048312128	0.019723407	0.172978909	0.06220716	-0.41905017
ES	0.426919556	0.259878689	-0.007215665	-0.131144367	0.25121175	-0.129629771	0.403591724
EE	0.038959782	-0.113710926	-0.227904855	0.339050813	-0.133303008	-0.064748891	-0.201696035
IN	-0.469863359	-0.267676841	-0.188020288	-0.317994993	0.412756086	0.253021631	0.111955286
IO	-0.122814373	0.056652546	-0.125361847	0.03792394	0.095742055	-0.182302581	0.359575483
FAr	-0.057154891	0.014271255	0.303625758	-0.210415667	-0.567849769	-0.191700829	-0.00332367
TBLr	-0.266800377	0.279797707	0.228807449	-0.14978157	0.145371473	-0.238209022	-0.413156342
AGr	0.069118103	0.008227511	-0.004079106	-0.18336757	-0.303043068	0.52714368	0.090386865
SPLr.l	0.178898959	0.359957261	-0.462995625	-0.293841774	0.004054536	0.173258047	-0.103413131
IFLr.l	-0.352501894	-0.193922056	0.002786908	0.157568267	0.04772871	-0.046397338	-0.113553524
MO	-0.000619521	-0.057816313	0.271775541	0.090085724	0.002030737	-0.003433238	0.40989474
IOS	-0.02790961	0.257813239	0.336314946	0.217437021	-0.064780745	0.352986111	0.03220845
V	-0.010841335	0.086524979	0.460243812	-0.002655612	0.309267431	0.129809552	-0.119165889
T4r.l	0.091314647	-0.276112686	0.005762572	0.333431445	-0.07395688	0.301989669	0.038025431
	PC15	**PC16**	**PC17**	**PC18**	**PC19**	**PC20**	**PC21**
Standard deviation	0.376199721	0.365477475	0.339179752	0.282916626	0.236187037	0.171149685	0.149480188
Proportion of Variance	0.00674	0.00636	0.00548	0.00381	0.00266	0.00139	0.00106
Cumulative Proportion	0.97923	0.98559	0.99107	0.99488	0.99754	0.99894	1
eigen	0.14152623	0.133573785	0.115042904	0.080041817	0.055784316	0.029292215	0.022344327
SVL	-0.083499418	0.052323475	-0.106015238	0.003344968	-0.031996278	0.03916795	-0.019457555
BW	-0.178247777	-0.058497317	0.597846907	-0.255954135	0.379366422	-0.199217481	0.221418054
HL	0.08265144	-0.13083192	-0.024132742	0.014837636	0.131660321	0.479388229	0.2891919
HW	0.244724903	-0.059011694	0.113956101	-0.074931386	0.04577337	-0.069612504	-0.732492773
HD	0.050630806	0.316234183	0.16660119	0.299515683	-0.49781424	0.046244883	0.219619924
EL	0.296740909	0.376559691	-0.260809485	-0.089676204	-0.027290387	-0.071210438	0.140852117
ED	-0.231235089	-0.168155577	-0.115371244	0.284151752	0.361474786	0.202917841	-0.049017145
EN	0.022603597	0.420837275	-0.236893263	0.02900181	0.436157574	0.238920063	-0.045962367
ES	0.248024801	-0.355459446	-0.140327076	0.068202164	0.014098586	-0.168841736	0.325882917
EE	-0.393037351	-0.425564191	-0.418495874	-0.066368342	-0.173649977	-0.04666024	-0.080329

	PC15	PC16	PC17	PC18	PC19	PC20	PC21
IN	-0.081626216	0.152259274	-0.192312604	-0.041856001	0.036286384	-0.306664296	0.054524547
IO	-0.115305892	0.069986307	0.310505175	0.23842857	-0.096262685	0.459593261	-0.180632462
FAr	-0.116757716	0.126720851	0.055407627	0.30600813	0.081267972	-0.470097472	-0.064137815
TBLr	-0.021197578	-0.178071358	0.100589652	-0.310374261	-0.331807871	0.142300685	0.047913831
AGr	0.334353255	-0.016725971	-0.026314358	-0.397995653	-0.105887039	0.100382932	-0.137085205
SPLr.l	-0.184269598	0.036899566	0.194841372	0.163667167	-0.204455175	-0.121267207	-0.156153276
IFLr.l	0.428551026	-0.165166993	0.155004291	0.142458525	0.008969802	-0.065554124	0.111490515
MO	-0.391157106	0.255079755	-0.004834981	-0.342258191	-0.175496507	0.081476947	0.028734251
IOS	-0.059743664	-0.04851249	0.20733712	-0.095515858	0.057436279	-0.015186946	0.173384569
V	0.002458015	-0.062420243	-0.039680685	0.361422477	-0.145522506	0.02608135	0.040083497
T4r.l	0.117972701	-0.206198025	0.0622901	0.17118706	0.012651195	0.069479213	-0.122746338

Table 6. Summary statistics of normalized morphometric and meristic characters among the *Dixonius* species.

Species	SVL	BW	HL	HW	HD	EL	ED	EN	ES	EE	IN	IO	FAr	TBLr	AGr	SPLr.l	IFLr.l	MO	IOS	V	T4r.l
***Dixonius fulbrighti* sp. nov. (N=4)**																					
Mean	1.55	0.85	1.03	0.81	0.54	0.05	0.33	0.45	0.55	0.49	0.11	0.21	0.66	0.75	1.18	8	6.25	6	8.25	23	14
SD	0.083	0.033	0.009	0.037	0.049	0.068	0.017	0.033	0.087	0.033	0.007	0.015	0.032	0.032	0.020	0.408	0.645	0	0.5	0.816	0.408
Lower	1.48	0.81	1.02	0.76	0.49	-0.03	0.31	0.41	0.44	0.44	0.11	0.19	0.62	0.72	1.16	7.5	5.5	6	8	22	13.5
Upper	1.66	0.88	1.04	0.84	0.61	0.13	0.35	0.48	0.62	0.52	0.12	0.22	0.70	0.80	1.20	8.5	7	6	9	24	14.5
***D. gialaiensis* (N=3)**																					
Mean	1.62	0.93	1.07	0.89	0.73	0.04	0.47	0.50	0.63	0.51	0.14	0.11	0.75	0.83	1.23	7.5	6.33	6	7	20.33	14.17
SD	0.060	0.007	0.003	0.001	0.007	0.005	0.002	0.007	0.002	0.005	0.018	0.029	0.034	0.013	0.025	0.5	0.577	0	0	1.155	0.289
Lower	1.56	0.92	1.06	0.89	0.72	0.04	0.47	0.49	0.63	0.51	0.12	0.08	0.73	0.82	1.20	7	6	6	7	19	14
Upper	1.68	0.93	1.07	0.89	0.73	0.05	0.47	0.50	0.64	0.52	0.15	0.13	0.79	0.84	1.25	8	7	6	7	21	14.5
***D. lao* (N=3)**																					
Mean	1.67	0.98	1.11	0.94	0.71	0.15	0.52	0.57	0.69	0.57	0.18	0.16	0.79	0.87	1.29	9	7.83	7.5	8.33	23.33	15
SD	0.099	0.014	0.017	0.005	0.013	0.022	0.016	0.042	0.028	0.012	0.013	0.047	0.017	0.007	0.003	0.5	0.289	0.5	0.577	0.577	0
Lower	1.55	0.96	1.09	0.93	0.70	0.12	0.51	0.54	0.67	0.56	0.17	0.12	0.78	0.86	1.29	8.5	7.5	7	8	23	15
Upper	1.74	0.99	1.13	0.94	0.72	0.17	0.54	0.62	0.72	0.58	0.20	0.21	0.81	0.87	1.29	9.5	8	8	9	24	15
***D. minhlei* (N=6)**																					
Mean	1.65	0.97	0.86	0.82	0.67	0.12	0.45	0.55	0.68	0.56	0.17	0.57	0.80	0.86	1.33	7.75	6.42	5.83	7.67	21.33	14.33
SD	0.025	0.012	0.008	0.037	0.022	0.044	0.022	0.021	0.025	0.022	0.023	0.025	0.020	0.017	0.060	0.418	0.376	0.258	1.211	1.366	1.033
Lower	1.61	0.95	0.85	0.79	0.65	0.08	0.42	0.52	0.64	0.53	0.14	0.53	0.78	0.85	1.28	7	6	5.5	7	20	13
Upper	1.68	0.98	0.87	0.89	0.71	0.18	0.49	0.57	0.70	0.59	0.21	0.60	0.83	0.89	1.44	8	7	6	10	23	15
***D. muangfuangensis* (N=3)**																					
Mean	1.69	1.03	1.15	0.99	0.78	0.21	0.47	0.54	0.68	0.66	0.19	0.33	0.78	0.81	1.35	8.17	6.5	6	10	22.33	13.5
SD	0.096	0.001	0.016	0.006	0.005	0.031	0.030	0.003	0.011	0.005	0.011	0.006	0.004	0.001	0.032	0.289	0.5	0	0	0.577	1.323
Lower	1.58	1.03	1.13	0.97	0.77	0.18	0.44	0.54	0.67	0.65	0.18	0.32	0.78	0.81	1.31	8	6	6	10	22	12
Upper	1.75	1.03	1.16	0.99	0.78	0.25	0.50	0.54	0.69	0.66	0.20	0.34	0.78	0.81	1.38	8.5	7	6	10	23	14.5
***D. siamensis* (N=8)**																					
Mean	1.62	0.93	1.09	0.90	0.70	0.13	0.43	0.54	0.68	0.58	0.25	0.54	0.80	0.84	1.27	7.94	6.56	5.94	9.63	20.63	14.38
SD	0.077	0.0357	0.009	0.014	0.029	0.048	0.034	0.044	0.021	0.031	0.046	0.016	0.028	0.017	0.021	0.320	0.496	0.177	0.518	1.061	0.835
Lower	1.61	0.95	0.85	0.79	0.65	0.08	0.42	0.52	0.64	0.53	0.14	0.53	0.78	0.85	1.23	7	6	5.5	7	20	13
Upper	1.68	0.987	0.87	0.89	0.71	0.18	0.49	0.57	0.70	0.59	0.21	0.60	0.83	0.89	1.44	8	7	6	10	23	15
***D. somchanhae* (N=6)**																					
Mean	1.62	0.97	1.07	0.91	0.70	0.17	0.46	0.48	0.65	0.50	0.18	0.23	0.73	0.83	1.26	7.75	5.67	6	8.17	23.33	14.67
SD	0.045	0.033	0.013	0.009	0.026	0.034	0.043	0.041	0.024	0.023	0.073	0.150	0.050	0.016	0.027	0.418	0.408	0	0.983	1.633	1.033
Lower	1.55	0.93	1.05	0.90	0.66	0.10	0.40	0.42	0.62	0.47	0.098	0.13	0.68	0.80	1.24	7	5	6	7	21	13
Upper	1.67	1.01	1.08	0.92	0.74	0.20	0.51	0.55	0.68	0.54	0.29	0.53	0.82	0.84	1.31	8	6	6	10	26	16
***D. vietnamensis* (N=12)**																					
Mean	1.56	0.83	0.83	0.82	0.65	-0.03	0.41	0.47	0.61	0.48	0.10	0.29	0.67	0.78	1.20	6.75	6.29	5.63	8.67	19.25	13.25
SD	0.088	0.055	0.016	0.028	0.049	0.054	0.030	0.031	0.035	0.055	0.070	0.165	0.063	0.035	0.051	1.177	0.450	0.377	0.985	1.545	0.399
Lower	1.41	0.72	0.80	0.77	0.58	-0.13	0.35	0.42	0.55	0.36	0.02	0.12	0.55	0.73	1.12	5	6	5	7	15	12.5
Upper	1.66	0.92	0.85	0.86	0.72	0.05	0.46	0.51	0.68	0.55	0.28	0.53	0.76	0.85	1.27	8	7	6	10	21	14

(V) along PC2 (Table 5). The PCA analysis indicates that the population from Duc Co District, Gia Lai Province is widely separated from all other species along the ordination of the first two axes except for the distantly related *D. vietnamensis*. Moreover, the new species is well-separated from all other species in the DAPC except for slight overlap with *D. minhlei* in their 67% inertia ellipses (Fig. 4B).

The MFA analysis recovered all species being separated from each other, including the population from Duc Co District, Gia Lai Province and its close relative *D. gialaiensis* and *D. minhlei* (Fig. 5A). The new population plots closest to the distantly related *D. vietnamensis*. The morphometric data contributed to approximately 40% of the 31.6% of the variation along Dim-1, followed by the categorical and meristic data. For Dim-2, the categorical data contributed 80% of the 17.8% of the variation, followed by morphometric and meristic data. For Dim-3, the meristic data contributed 70% of the 12.0% of the variation, followed by morphometric and categorical data. For Dim-4, the meristic data contributed 50% of the

8.5% of the variation, followed by categorical and morphometric data (Fig. 5B).

The ANOVAs and subsequent TukeyHDS tests demonstrated that the population from Duc Co District, Gia Lai Province bears statistically different mean values from all other species in various combinations of characters (Tables 6, 7) and differs significantly from the closely related *D. gialaiensis*, in head length (HL), head width (HW), head depth (HD), eye diameter (ED), and tibia length (TBL) (Fig. 7; Tables 6, 7). Given that we had only one specimen of the closely related *D. minhlei* we could not conduct any statistical analyses including it. However, the two do differ discretely in head length (HL), body width (BW), head depth (HD), eye diameter (ED), eye nostril distance (EN), eye snout distance (ES), forearm length (FA), tibia length (TBL), and axilla to groin length (AG) as well as in color pattern of two rows of regularly disposed whitish tubercles on the dorsal surface in the Duc Co District, Gia Lai Province population as opposed to their absence in *D. minhlei*. Variation in all metric characters is visualized in Figs 6, 7.

Given the phylogenetic placement of the population from Duc Co District, Gia Lai Province not being embedded within any other species, its statistically different mean values in a number of morphometric and meristic characters among the other species, and its generally isolated morphospatial placement in all three morphometric analyses, we consider this population to be an evolutionarily unique and statistically diagnosable lineage and therefore describe it below as a new species.

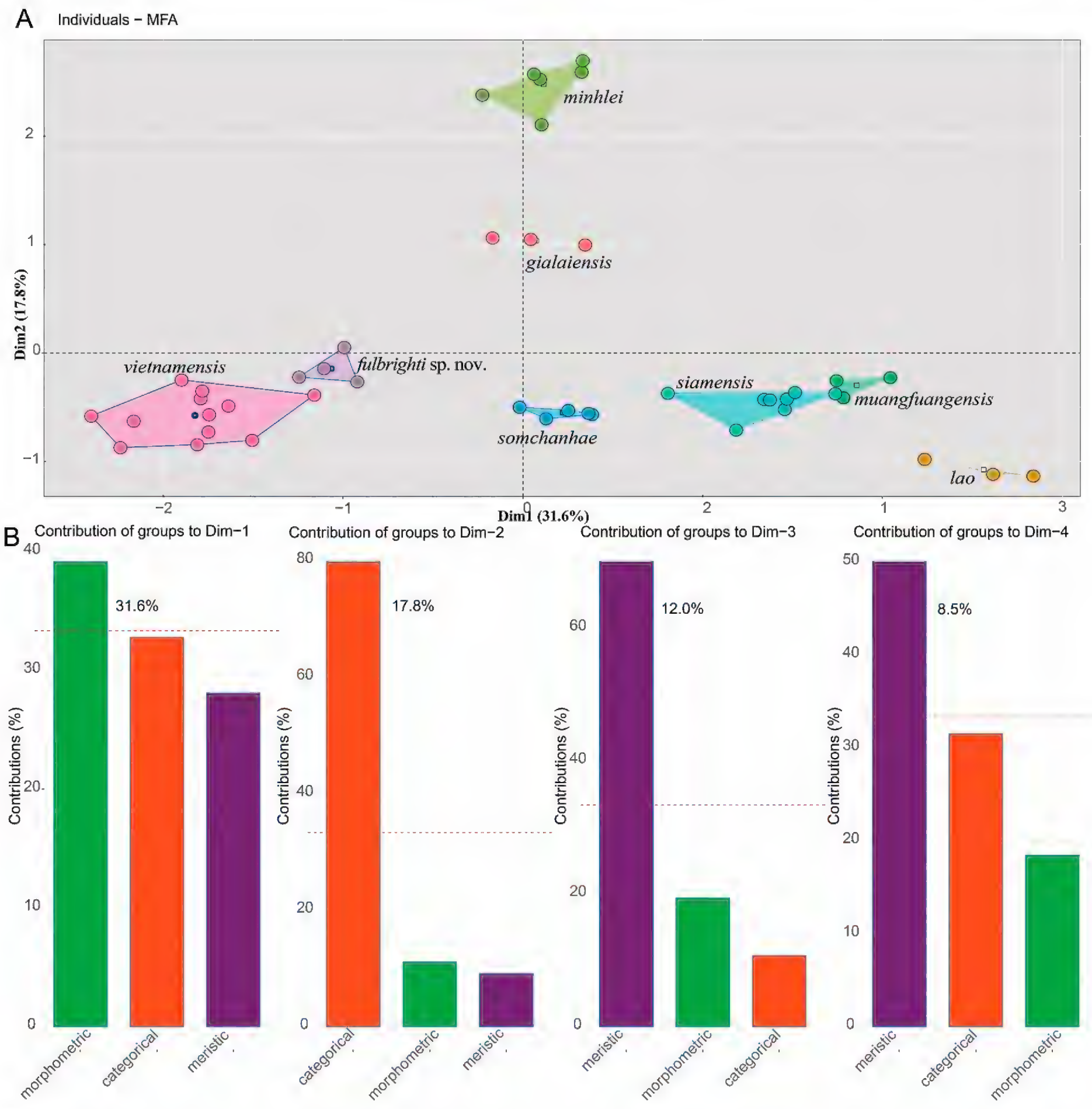

Figure 5. A. MFA scatter plot based on the total evidence data set showing the morphospatial relationships among the *Dixonius* species. **B.** Bar graphs showing the percent contribution of each data type to the overall variation in the data set. The dashed red line in the bar graphs indicates the expected average value if the contributions of each data type were equal.

Table 7. Significant *p*-values from the results of the ANOVA and TukeyHDS analyses comparing all combinations of species pairs of *Dixonius*. Character abbreviations are listed in the Materials and methods.

Morphometric characters	BW	HL	HW	HD	EL	ED	EN	ES	FAr	TBLr	AGr	IFLr.l	MO	V
D. fulbrighti sp. nov. vs. *gialaiensis*		0.021	0.001	<0.001		<0.001				0.006				
D. fulbrighti sp. nov. vs. *lao*	0.002	<0.001	<0.001	<0.001		<0.001	<0.001	<0.001	0.005	<0.001	0.018	0.002	<0.001	
D. fulbrighti sp. nov. vs. *minhlei*	<0.001	0.00		<0.001		<0.001	0.001	<0.001	<0.001	<0.001	<0.001			
D. fulbrighti sp. nov. vs. *muangfuangensis*	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	0.016	0.001	0.013		<0.001			
D. fulbrighti sp. nov. vs. *siamensis*	0.016	<0.001	<0.001	<0.001		<0.001	0.002	<0.001	<0.001	<0.001	0.012			
D. fulbrighti sp. nov. vs. *somchanhae*	<0.001	0.017	<0.001	<0.001	0.013	<0.001		0.017		0.003	0.032			
D. fulbrighti sp. nov. vs. *vietnamensis*		0.00		<0.001		<0.001								<0.001

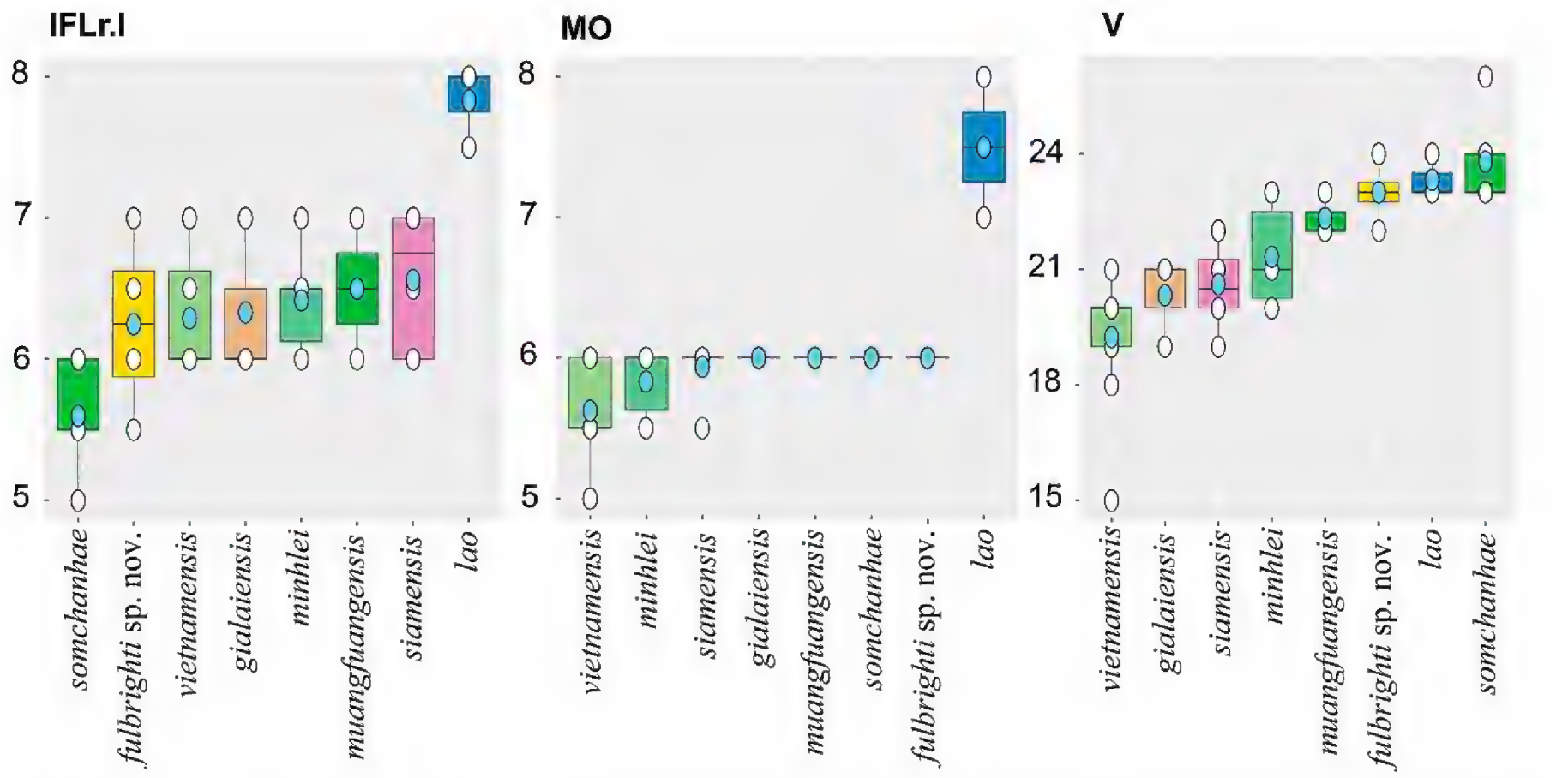

Figure 6. Boxplot comparisons of significantly different meristic characters among the *Dixonius* species. Light-blue ellipses are means and the black horizontal bars are medians.

Taxonomy

Dixonius fulbrighti sp. nov.

https://zoobank.org/F9F5907A-1B5D-4985-B6FE-8B4092947C56

Fulbright Leaf-toed Gecko, Fig. 8

Type material. ***Holotype.*** Adult male, VNUF R.2022.81 (Field no. GL22.01) in Grong Village, Ia Krieng Commune, Duc Co District, Gia Lai Province (13°44'25.6"N, 107°43'39.5"E; 372 m a.s.l.), collected by Vinh Quang Luu, Tron Thanh Tran, Siu Biu, Ksor Lang on 8 July 2022.

Paratypes. VNUF R.2022.82 (Field GL22.02), adult female, VNUF R.2020.83 (Field No. GL22.03), juvenile female, VNUF R.2020.84 (Field No. GL22.04), juvenile female; bear the same data as the holotype.

Diagnosis. *Dixonius fulbrighti* sp. nov. can be separated from all other species of *Dixonius* by possessing the unique combination of having a maximum SVL of 46.0 mm; 16–20 longitudinal rows of dorsal tubercles at midbody; 22–24 longitudinal rows of ventrals across the abdomen; seven–nine supralabials, sixth in at midorbital position; five–seven infralabials; eight or nine interorbital scales; seven precloacal pores in the adult male, femoral pores lacking; seven precloacal-pitted scales, femoral pores absent in adult female; 13–15 lamellae on fourth toe; dorsal ground color grey-brown with the presence of thick, irregular-shaped, black brown blotches from head to body; canthal stripe extending from the nostrils continuing behind orbit to back of head; dark bars on the lips absent; two rows of regularly disposed whitish tubercles along the flanks to originale portion of tail. These characters are scored across all *Dixonius* species from Vietnam and Laos in Tables 6 and 7.

Description of the holotype. Adult male, SVL 46.0 mm; head moderate in length (HL/SVL 0.30), wide (HW/HL 0.57), depressed (HD/HL 0.42), distinct from neck; prefrontal region concave; canthus rostralis rounded; snout elongate (ES/HL 0.35), rounded in dorsal profile; eye moderate size (ED/HL 0.16); ear opening oval, obliquely oriented, moderate in size; diameter of eye smaller than eye to ear distance (ED/EE 0.59); rostral rectangular, partially divided dorsally by straight rostral groove, bordered posteriorly by large left and right supranasals, bordered laterally by first supralabials; external nares bordered anteriorly by rostral, dorsally by large supranasal, posteriorly by two smaller postnasals, bordered ventrally by first supralabial; 8,8 (R,L) rectangular supralabials extending to below and slightly past posterior margin of eye, sixth in midorbital position; 6,7 (R,L), infralabials tapering smoothly to just below midpoint of eye, decreasing gradually in size; scales of rostrum and lores flat to domed, larger than granular scales on top of

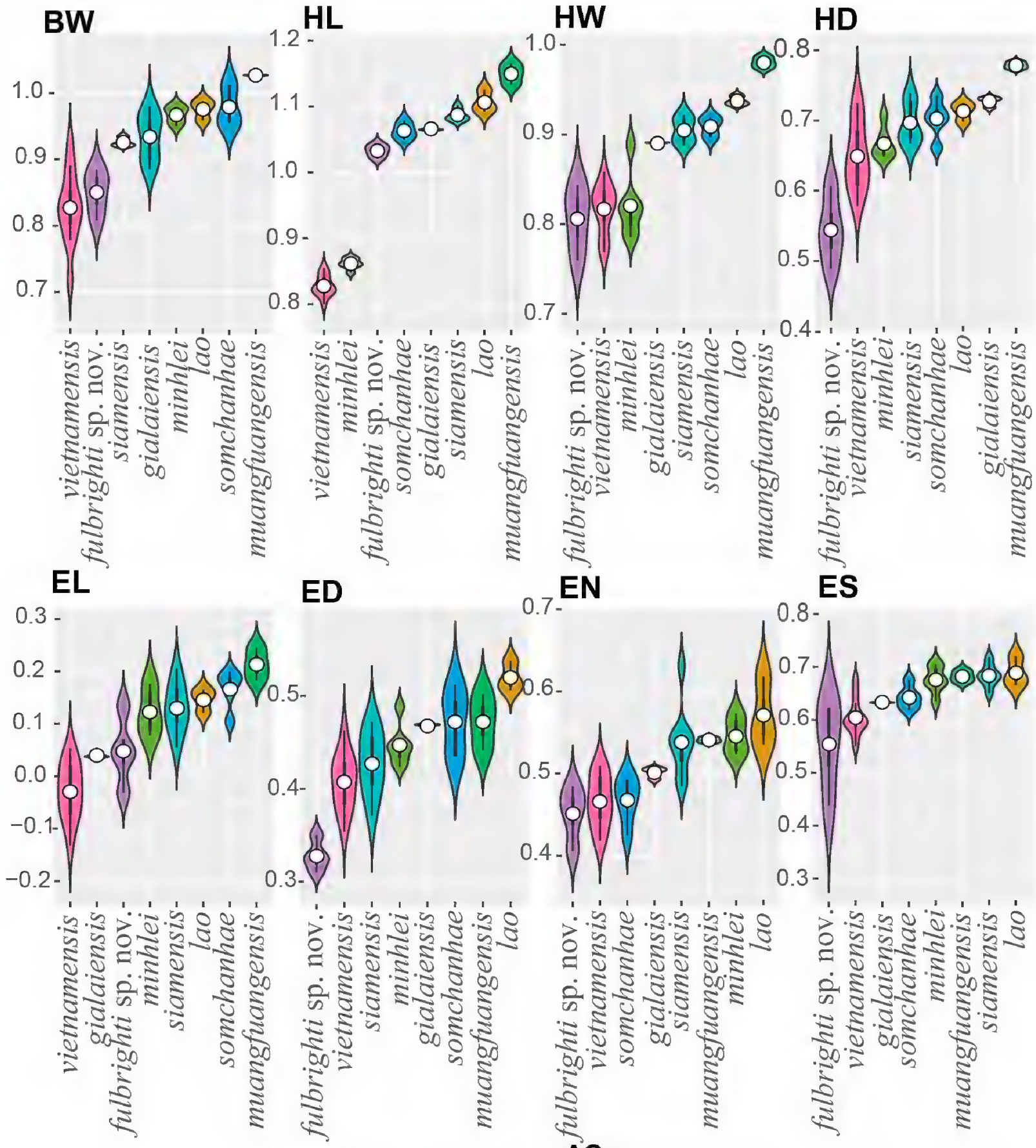

Figure 7. Violin plots of the significantly different morphometric characters overlain with box plots showing the range, frequency, mean (white circle), and 50% quartile (black rectangle) of the size-adjusted morphometric characters.

head and occiput; scales of occiput intermixed with distinct, small, conical tubercles; superciliaries elongate, largest anteriorly; mental triangular, bordered laterally by first infralabials and posteriorly by two postmentals (large right trapezoidal shape and left irregular shape) contacting medially for 60% of their length posterior to mental; gular and throat scales small, granular, grading anteriorly into slightly larger, flatter, smooth, imbricate, pectoral and ventral scales.

Body relatively short (AG/SVL 0.44); dorsal scales small, granular interspersed with larger, conical, regularly arranged, keeled tubercles; tubercles extend from top of head onto posterior half of original tail forming longitudinal rows, terminating at last portion of orginale tail; smaller tubercles extend anteriorly onto nape and occiput, diminishing in size and distinction on top of head; 18 longitudinal rows of tubercles at midbody; 32 paravertebral scales, number of scales in a paravertebral row from first scale posterior to parietal scale to last scale at the level of vent opening; 22 paravertebral scales in a row between limb insertions; 24 flat, imbricate, ventral scales much larger than dorsal scales; 7 enlarge, pore-bearing, precloacal scales in an angular series; and no deep precloacal groove or depression.

Forelimbs moderate in stature, relatively short (FA/SVL 0.12); granular scales of forearm slightly larger than those on body, interspersed with small tubercles; hind limbs more robust than forelimbs, moderate in length (TBL/SVL 0.15), covered dorsally by granular scales interspersed with large, and small conical tubercles; ventral scales of thigh flat, imbricate, larger than dorsals; subtibial scales flat, imbricate; proximal femoral scales smaller than distal femorals; femoral pores absent; digits relatively long with 14 lamellae on fourth toe; and claws well developed.

Regenerated tail 46.4 mm in length, 5.4 mm in width at base, tapering to a point; dorsal scales of tail flat, oval with conical, keeled tubercles in anterior part; median row of transversely expanded subcaudal scales, significantly larger than dorsal caudal scales on original portion; base of tail bearing hemipenal swellings; and postcloacal scales flat, imbricate.

Coloration in life. (Fig. 8) Dorsal ground color of grey-brown with thick, irregular-shaped, black to brown blotches extending from head to the body; canthal stripe extending from behind the nostrils conitinuing behind orbit to back of head; dark bars on lips; uneven light-colored spots running from postorbital region along the flanks to the base of tail; dorsal surface of fore- and hind limbs uniformly light-grey with dark-brown spots; dorsum of orginal tail covered with some small black to brown blotches; all ventral surfaces uniformly beige.

Variation. (Fig. 8) The female paratypes (VNUF R.2022.82–84) are generally uniformly brown dorsally with uneven light-colored spots running from the postorbital region along the flanks to the tail tip; dorsum of posterior part of tail grey with narrowed light-colored bands. Additional measurements are in Tables 2, 3, 8.

Distribution. *Dixonius gialaiensis* sp. nov. is currently known only from the type locality of in Grong Village, Ia Krieeng Commune, Duc Co District, Gia Lai Province, Central Highlands, Vietnam (Fig. 1).

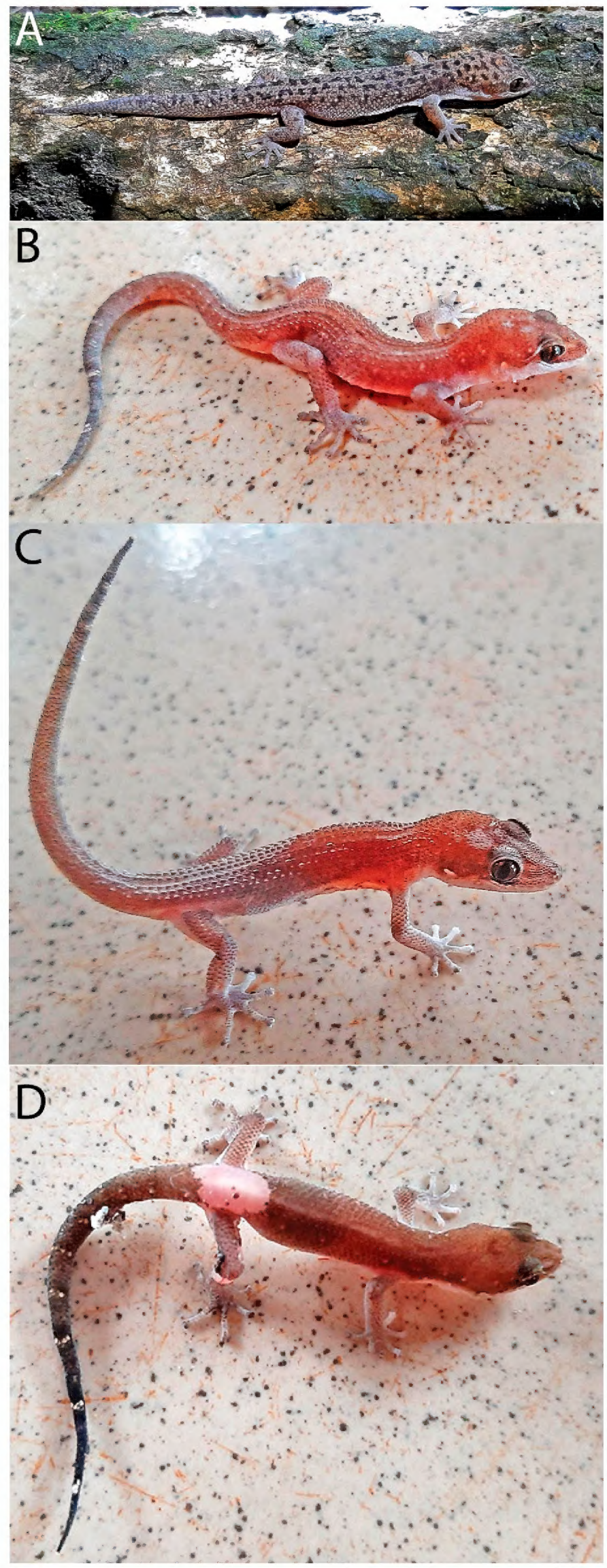

Figure 8. Dorsal views of *Dixonius fulbrighti* sp. nov. **A.** Adult male holotype VNUF R.2022.81 (Field no. GL01); **B.** Adult female paratype VNUF R.2022.82 (Field No. GL02); **C.** Juvenile female paratype VNUF R.2022.83 (Field no. GL03); **D.** Juvenile female paratype VNUF R.2022.4 (Field no. GL04) in Grong Village, Ia Krieng Commune, Duc Co District, Gia Lai Province.

Natural history. The specimens were found on the forest floor, during the evening between 1900 hrs and 2000 hrs. The surrounding habitat consisted of secondary forest with Narra Padauk (*Pterocarpus macrocarpus*)

trees as the dominant species and shrub trees on the forest floor (Fig. 9). Currently, the area is managed by the local commune, but it is not part of any protected area system. Therefore, further investigation on the population status is needed to suggest appropriate conservation measures.

Etymology. The new species is named after Mr. J. William Fulbright, the founder of the Fulbright Program which has provided opportunities for thousands of students, scholars, and professionals from around the world to study, teach, and conduct research in the United States and other countries in order to promote a greater understanding and cooperation between nations.

Comparisons. *Dixonius fulbrighti* sp. nov. is the sister species to a clade containing *D. gialaiensis* and *D. minhlei* (Fig. 2). It differs from the former by an uncorrected pairwise sequence divergence of 3.12% (Table 4) and significantly normalized morphometric characters, including a significantly lower mean head length (HL) (1.03 vs. 1.07, p = 0.021), head width (HW) (0.81 vs. 0.89, p = 0.001), head depth (HD) (0.54 vs. 0.73, p = <0.001), eye diameter (ED) (0.33 vs. 0.47, p = <0.001), and tibia length (TBL) (0.75 vs. 0.83, p = <0.006). In addition, it differs from *D. gialaiensis* in dorsal color pattern of head and body (thick, irregular-shaped, back brown blotches versus disseminated round black brown blotches). It differs from the latter by 3.6% genetic difference (Table 4). Additionally, the new species can be distinguished from *D. minhlei* by having a significantly higher mean number of head length (HL) (1.03 vs. 0.86, p = 0.000), a significantly lower mean number of body width (BW) (0.85 vs. 0.97, p = <0.001), head depth (HD) (0.54 vs. 0.67, p = <0.001), eye diameter (ED) (0.33 vs. 0.45, p = <0.001), eye nostril distance (EN) (0.45 vs. 0.55, p = <0.001), eye snout distance (ES) (0.55 vs. 0.68, p = <0.001), forearm length (FA) (0.66 vs. 0.80, p = <0.001), tibia length (TBL) (0.75 vs. 0.86, p = <0.001), and axilla to groin length (AG) (1.18 vs. 1.33, p = <0.001). Additionally, the new species differs from *D. minhlei* by the presence of two regularly disposed whitish tubercles on each side of body (absent in *D. minhlei*). Statistically significant differences among *D. fulbrighti* sp. nov. and all other species and populations are presented in Tables 5–7.

Figure 9. Habitat of *Dixonius fulbrighti* sp. nov. in Grong Village, Ia Krieng Commune, Duc Co District, Gia Lai Province

Table 8. Measurements (in mm) and morphological characters of the type series of *Dixonius fulbrighti* sp. nov. (forviations see material and methods). Measurements taken on right side; SPL/IFL/MO/T4 given in right/ left order; * tail regenerated.

Character	VNUF R.2022.81 (Holotype)	VNUF R.2022.82 (Paratype)	VNUF R.2022.83 (Paratype)	VNUF R.2022.84 (Paratype)
Sex	**Adult Male**	**Adult female**	**Juvenile Female**	**Juvenile Female**
SVL	46	35.2	31.1	30.1
TaL	46.4*	50.3	38.4*	33.5
TW	5.4	3.8	3	3.7
BW	10.7	7.5	5.1	5.6
AG	20.2	14.2	12.5	13.5
HL	13.7	10.9	9.2	9.3
HW	7.8	6.9	5.1	5.8
HD	5.7	3.3	2.4	3
EL	1.4	1.1	1.2	0.8
TBL	6.8	6.2	5.1	4.6
FA	5.5	4.7	4.5	3.7
ED	2.2	2.1	2.2	2
EN	3.4	2.9	2.3	2.7
ES	4.8	4.1	2.3	3.4
EE	3.7	3.2	2.5	2.9
IN	1.7	1.3	1.1	1.1
IO	1.9	1.6	1.4	1.5
V	24	23	23	22
DTR	18	16	19	20
PV	32	39	36	38
PV'	22	24	24	25
T4	14/14	15/14	14/14	14/13
IOS	9	8	8	8
ICS	28	24	26	24
SPL	8/8	8/7	8/8	9/8
IFL	6/7	6/5	7/7	6/6
MO	6	6	6	6
PP	7	7 (pitted scales)	0	0

Discussion

Dixonius fulbrighti sp. nov. is most closely related to the sister species *D. minhlei* and *D. gialaiensis*, but can be distinguished from the both species by differences in body shape and color pattern (see Table 5). The molecular analysis revealed a 3.12–3.61% uncorrected pairwise genetic distance between *Dixonius fulbrighti* sp. nov. and *D. gialaiensis* and *D. minhlei*, and all evolved separately in geographically isolated regions. The type locality of *D. fulbrighti* sp. nov. is approximately 55 km west of the type locality of *D. gialaiensis*, and the two areas are separated by fragmented habitats bearing residential areas and transport systems, including the National Road AH17, and the Local Roads DT663 and DT6754 (Fig. 1). Moreover, the type locality of *Dixonius fulbrighti* sp. nov. is approximately 280 km northeast of the type locality of *D. minhlei*, and these two localities are isolated by different habitat types and river systems. Additionally, the divergence time estimated by the BEAST analysis indicates that *Dixonius fulbrighti* sp. nov. and the *D. minhlei* – *D. gialaiensis* clade have been evolving independently for a considerable period of time, approximately 4.12 million years ago. This is likely due to their historical isolation from each other in geographically distinct regions, that is maintained by current barriers to gene flow as well as by human activities such as road construction and deforestation, helping to maintain their separation. The fragmented habitats between the type localities of the these species suggest these barriers are continuing to impact the distribution and evolution of geckos in this region (Grismer et al. 2013; Epps and Keyghobadi 2015). Further research on the biogeography and genetic diversity of these species will be important for understanding their evolutionary history and informing conservation efforts to protect their unique habitats.

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